



# TOXICITY OF METALS SUCH AS IRON, NICKEL AND COBALT ON SEEDLINGS OF BROAD BEANS (*Vicia faba* L.)

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## ABSTRACT

Increased industrialization in developing countries like India has resulted into the enormous deterioration of air, water and soil. Presence of heavy metals causes bioaccumulation affecting the entire ecosystem and poses harmful consequences. On treating beans with increased concentration (10mM, 50mM, 100mM, 150mM) of heavy metals (Ni, Co, Fe) significant decrease ( $p \leq 0.05$ ) in seed germination, root length, shoot length, root and shoot length and dry weight was observed but there was significant increase ( $p \leq 0.05$ ) in phosphatase activity (acid and alkaline phosphatase), protein and phenolics content. The metal toxicities of various metal studied was  $\text{Co} > \text{Ni} > \text{Fe}$ . The present study concludes that the Broad Beans (*Vicia faba* L.) is a metal resistant plant which has devised various mechanism such as de novo synthesis of anti-stress protein or mitigation of ROS by enhanced phenolic production to combat metal stress.

**KEYWORDS:** Ni, Fe, Co, toxicity, phosphatase, amylase

## INTRODUCTION

Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. Heavy metals are essential for plant growth and are main components of many vital compounds but when they are present in increasing concentrations, it leads to growth delay and inhibition of biochemical reactions (Asati et al., 2016). Heavy metal stress may also stimulate the formation of free radicals and reactive oxygen species, resulting in oxidative stress (Naguib, 2016).

Cobalt is a heavy metal occurring naturally in different chemical forms in our environment. It is an essential nutrient for plant and animals at lower concentration but becomes toxic at higher concentration. The level of toxicity depends on plant species, soil type and soil chemistry (Hasan et al., 2011). Nickel is a group alpha transition metal which causes physiological alterations and toxicity symptoms such as chlorosis and necrosis (Negi et al 2014). Iron is the major constituent essential for plants and has many biological roles like photosynthesis, chloroplast development and chlorophyll biosynthesis. The excess of iron causes free radical production that irreversibly impairs cellular structure and damages membrane, DNA and protein (De Dorlodot et al., 2005).

The **Broad Beans (*Vicia faba* L.)** is an important component of food having high nutritional quality. The Ni, Co, Fe are micronutrients which are essential for the plant in low concentration. So the present study was done to analyse the effect of some these heavy metals (Ni, Co, Fe) with different concentrations in the germination, protein content and enzymatic status in beans.

## MATERIALS AND METHODS

Seeds were purchased from local markets and were sterilized in 2% bevestin to avoid fungal infection. Seed germination was carried out in glass petri plates. Each dish contained 10mL of different concentration (10mM, 50mM, 100mM, 150mM) of metal (Ni, Co, Fe) and 10mL of distilled water (control). In vitro germination was designed for three replicates and all growth parameters (no. of seeds germinated, root length, shoot length, root/shoot length) were recorded at different time intervals. The germinated seeds were homogenized in distilled water, pH 7. The homogenates were centrifuged at 5000g for 10 minutes at 4°C and the supernatant was used as enzyme sample. The protein estimation was done as described by Lowry et al (1951). Total protein was expressed as mg of protein hydrolysed per mg protein. The total phenolics was estimated by modified Folin-Ciocalteu method and spectrophotometrically measured at wavelength 760nm. Acid and alkaline phosphatase activity was measured by protocol given by Mishra and Dubey (2008). Amylase activity was measured as described by Johnson (2007).

**Statistical analysis:** Statistical analysis was based on one-way analysis of variance (ANOVA). The effects of heavy metal treatment were considered statistically significant when  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

There was significant decrease ( $p \leq 0.05$ ) in seed germination, root length, shoot length due to metal toxicity (Ni, Fe, Co). However, the plant was able to tolerate higher conc. of metals (up to 150mM) as Ni, Co and Fe are essential micronutri-

ents. The general decreasing order of root and shoot length is  $\text{Co} > \text{Ni} > \text{Fe}$ . Since Fe is mainly involved in the process of plant photosynthesis, chloroplast development and chlorophyll biosynthesis, Fe toxicity seems to have less effect on growth parameters as compared to Co and Ni. Ni affects amylase, protease and ribonuclease enzyme activity which retarded seed germination (Ahmad and Ashraf, 2011). The decrease in seed germination may be due to lowering of water potential or through ionic imbalance as Fe toxicity impairs the absorption of various other metals. The reduction in root length under heavy metal stress could be due to reduction in mitotic cell division in meristematic zone of roots. In the present study, these metals significantly affected plant height, root length, fresh and dry weight due to the digestion and mobilization of food reserves like proteins and carbohydrates in germinating seeds (Ashraf et al., 2011). The decrease in fresh weight could also be due to metal induced decline in tissue water content which significantly inhibited shoot vitality, preventing plant from absorbing inorganic nutrients and leading to inhibited plant growth (Singh et al., 2013).

There was a significant increase in protein content in beans treated with increasing conc. of Ni, Co, Fe (10mM, 50mM, 100mM, 150mM) ( $p \leq 0.05$ ). The ability to de novo synthesize stress proteins and peptides such as enzymes involved in Krebs cycle, glutathione and phytochelatin biosynthesis and some heat shock proteins could be plant strategy of avoiding uptake of these metals (Mishra et al., 2006). The Fe toxicity at higher conc. (100mM and 150mM) resulted in significantly higher levels of protein level than Ni and Co implying iron role in the mechanism of metal tolerance by making biochemical and structural adjustments that enable the plant to cope with stress conditions (Rout and Sahoo, 2012).

The Ni, Fe and Co at low conc. (10mM) cause significant increase in the amylase levels. However, at higher conc. (100mM, 150mM) there was significant decrease in the amylase activity as reported earlier by Mittal et al (2015). The decreased amylase activity interfere with the synthesis of sugar from starch either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient due to which the carbohydrates are not hydrolyzed in primary root and shoot which affects germination (Pena et al., 2011).

The **Broad Beans (*Vicia faba* L.)** has shown tolerance to Ni and Fe toxicity as evident from significantly higher level of acid phosphatase and alkaline phosphatase activity when grown in presence of Ni (10mM) and Fe (150mM). Similar results were reported in Ni resistant plants (Gabbriellini et al., 1989) and Al resistant plants (Huttová et al., 2002). Higher acid phosphatase activity could be due to the ability of the resistant Broad bean plant used in our study to maintain a relatively high  $\text{Mg}^{2+}$  concentration inside the root tissues which play a major role in the modulation of acid phosphatase activity. However, there was no significant effect on acid and alkaline phosphatase activity with Co toxicity.

There was induction of phenol compounds synthesis at higher conc. of Ni (150 mM) as reported earlier by Diaz et al (2001). This could be due de novo synthesis of phenolics under heavy metal stress or increase in cell wall endurance due to the creation of physical barriers by lignin production by phenolics which deprevet cells against harmful action of heavy metals (Diaz et al 2001). The ability of the plant to tolerate metal stress could be due to the antioxidant action of phenolic compounds which have high tendency to chelate metals. In the present study, the high conc. of Fe (up to 150 mM) showed least toxicity could be due to

the presence of high levels of phenol which bind Fe through hydroxyl and carboxyl groups and may inactivate iron ions by chelating and additionally suppressing the superoxide-driven Fenton reaction.

It can be concluded that Broad Beans (*Vicia faba L.*) is a metal resistant plant which could tolerate high metal toxicity by enhancing the synthesis of phenolic compounds which prevent the formation of ROS due to its antioxidant activity and also by enhancing the activity of acid and alkaline phosphatase activity. Moreover out of Ni, Co and Fe, the most toxic metal is Cobalt and least is Fe which has several inherent mechanism of metal tolerance.

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